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PERMISSIVE EFFECT OF MOLSIDOMINE TOWARDS CARDIOPROTECTIVE ACTION OF ILOPROST IN MYOCARCIAL ISCHEMIA IN CATS

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Molsidomine, a donor of nitric oxide, is a drug used in the treatment of ischaemic heart disease. Iloprost, a stable analogue of prostacyclin, is a cardioprotective agent in dogs, cats and rats but not in men. We have studied an interaction between molsidomine and iloprost in protecting against consequences of the „no-reperfusion" myocardial ischaemia. In ten control open-chest cats the left descending coronary artery (LDCA) was ligated at a site of its branching. This procedure caused 80% of mortality and the survival time was 40.9 ± 8.6 min. The death of cats was preceded by continuous premature ventricular contractions (PVC) which appeared 2.1 ± 0.3 min after LDCA and occurred with frequency of 7.3 ± 0.6 per min. Molsidomine at a dose of 20 μg/kg i.v. given to ten cats before LDCA was neither cardioprotective nor it influenced the rate of mortality while iloprost at a dose of 2 μg/kg i.v. opposed the outcome of LDCA as alluded by the elongation of the survival time to 66.6 ± 7.6 min and the delay of the onset of PVC to 9.1 ± 1.9 min; also the frequency of PVC fell to 3.6 ± 0.4 per min, however, the LDCA-induced mortality (60%) was not significantly different from that in control animals (80%). On the other hand, in ten cats with LDCA which were pretreated with a mixture of molsidomine and iloprost there was observed a significant reduction of the LDCA-induced mortality (down to 20%) and a two fold increase in the survival time. Thereby, we conclude that molsidomine permitted enhancing the cardioprotective potency of iloprost. Indeed, a combination of molsidomine and iloprost granted a better cardioprotection than either iloprost alone or superoxide dismutase at a megadose of 3000 units/kg i.v.. The enhancement of cardioprotective potecy of iloprost by molsidomine was not accompanied by a synergism in the blood pressure lowering potencies of these drugs.

Key words: *molsidomine, iloprost, nitric oxide, prostacyclin, cardioprotection, myocardial ischaemia, superoxide dismutase*

INTRODUCTION

Nitric oxide (NO) is likely to represent the activity of "endothelium-derived relaxing factor" — EDRF (1). Nitric oxide shares with prostacyclin (PGI_2) its vasodilator, platelet-suppressant, cytoprotective and fibrinolytic properties, although cyclic-GMP rather than cyclic-AMP is responsible for these activities of NO (2—4). *In vitro* prostacyclin, prostaglandin E_1 or their synthetic

analogues were reported to synergize with nitric oxide or its donors in their capacities to protect against platelet aggregation (5—9) and ageing of leukocytes (7, 10) but they did not synergize in their vasorelaxant actions. (1, 7, 11). In patients with peripheral vascular diesease a concurrent administration of a NO-donor and a PGI_2-analogue elicited synergistic fibrinolytic effects (11). The enhancement by NO of the effects of PGI_2 on activated platelets, non-stimulated leukocytes and plasma fibrinolytic potential prompted us to study an interaction between these stimulators of guanylate and adenylate cyclases in protecting against consequences of myocardial ischaemia in anaesthetized open chest cats with ligated left descending coronary artery.

To this purpose molsidomine and iloprost the stabile substitutes of labile NO and PGI_2 were chosen. Molsidomine, N-ethoxy-carbonyl-3-morpholinosydnonimine, *in vivo* is metabolized to 3-morpholinosydnonimine (SIN-1), and this last gives rise to a direct donor of nitric oxide-N-morpholino-N-nitrosoaminoacetonitrile (SIN-1A) (3,12,13). Like NO (EDRF) (1—3) SIN-1 is a potent stimulator of soluble guanylate cyclase (14). Molsidomine along with organic nitrates is used for the treatment of ischaemic heart disease. Iloprost is a stable carbacyclin analogue of endogenous PGI_2. Iloprost and PGI_2 stimulate membrane-bound adenylate cyclase (15) through the same receptor sites. Although there are no reports on the effectiveness of iloprost in ischaemic heart disease in man, there are a few on the cardioprotective action of PGI_2 or iloprost in experimental myocardial ischaemia (16—18).

MATERIAL AND METHODS

Fifty three cats of both sex, body weight 2—3 kg were anaesthetized with sodium pentobarbitone (30 mg/kg i. p.). The chest was open along the sternum and breathing was supported with a respiratory pump (20 strokes per min, 20 ml of air per kg body weight). A silk thread was introduced under the left descending coronary artery, one milimeter below its branching from the left coronary artery. This procedure was described by Schrör et al (17). Our methodology differs from the original one in that a site of ligation of the left descending coronary artery is more close to the main trunk. In three sham-operated cats the thread was introduced under the artery and then left loose for 2 hours of observation. The remaining fifty cats were left for 15 minutes to recover from the immediate surgical stress and then, in groups of ten cats each, injected intravenously with saline (1 ml) or molsidomine (20 μg/kg) or iloprost (2 μg/kg) or a mixture of molsidomine (20 μg/kg) and iloprost (2 μg/kg) or superoxide dismutase (3000 units/kg). Fifteen minutes later the thread installed under the artery was tightened up and the ligature on the left descending coronary artery (LDCA) occluded its blood flow. In these cats mean arterial blood pressure (BP) was recorded from the right carotid artery while the first lead continuously monitored ECG in a typical tripolar system. The consequences of LDCA were measured as follows: frequency of premature ventricular contractions (PVC/min) which occured after LDCA; this was registered during 90 min in surviving cats or until the death of the rest of them; onset of PVC (To) was measured in minutes from the moment of LDCA and in this way survival time (Ts) was asessed; finally the mortality rate (M) was recorded in five groups of 10 cats each. The observation was carried out for 2 hours.

Statistics

Except for the mortality rate, all other measurements were presented as mean ± S. E., and statistical significance between the control group and treated groups of animals was calculated using unpaired Student's test at levels of $p = 0.05$ or $p = 0.01$. A difference between rates of mortality between control and experimental groups of animals was calculated using X2 test.

Drugs

The basic drugs used in this study had been kindly offerred by German pharmaceutical companies: molsidomine (Corvaton) by Casella-Riedel, iloprost (ZK 36 375) by Schering and superoxide dismutase (Peroxinorm) by Grünenthal.

RESULTS

In three sham-operated cats neither PVC nor a fall in BP occurred within 2 hours of observation. In the remaining 50 cats with the ligation of left descending coronary artery (LDCA) none type of pretreatment could prevent an acute fall in BP that occurred immediately following the occlusion. In ten control cats severe PVC were diagnosed in ECG as soon as 2 minutes after fastening of the ligature. These arrhythmias gradually increased in their severity and frequency (7.3 PVC/min) and ultimately led to the death of 80 percent of animals within 40 minutes or so (*Table 1*). This high rate of mortality

Table 1. The effect of saline or pharmacological treatment on premature ventricular contraction (PVC), onset of PVC (To), survival time (Ts), fall in arterial blood pressure (%FBP) and mortality rate (M) in anaesthised open chest cats after occlusion by ligation of their left descending coronary artery (LDCA). Except for the ratio M all other values are presented as mean ± S. E. Levels of statistical significance are * $p<0.05$ and ** $p<0.01$; @ — for statistical purposes 90 min. was taken as maximum Ts in survivals.

Treatment	Dose (i. v.)	FBP%	PVC/min	To	Ts(min)@	M
0.9% Saline	1 ml/kg	31±4	7.3±0.6	2.1±0.3	40.9±8.6	8/10
Molsidomine	20 µg/kg	32±5	7.3±0.9	3.0±0.4	55.2±7.8	7/10
Illoprost	2 µg/kg	28±4	3.6±0.4**	9.1±1.9**	66.6±7.6*	6/10
Molsidomine + Illoprost	2 µg/kg + 20 µg/kg	27±3	4.2±0.4**	6.6±0.6**	81.6±5.6**	2/10**
SOD	3000 µg/kg	29±4	6.1±0.7	2.3±0.3	64.5±7.2*	6/10

was diminished by none of pharmacological treatments except one, namely in the group of cats which were pretreated simultaneously with molsidomine and iloprost. In this group of cats not only the mortality rate dropped to 20 percent but also the survival time was doubled as compared with control. Molsidomine

given alone had no effect on any corollaries of LDCA. Iloprost manifested its cardioprotective action by decreasing the frequency of PVC, extending onset of PVC (To) and negligeably elongating survival time (Ts), however, mortality rate was not significantly lower. In the employed model of "no-reperfusion" myocardial ischaemia the pretreatment with superoxide dismutase hardly showed a cardioprotective effect. The only hint to its pharmacological activity was extension of the survival time. Immediate effects of slow intravenous injections of iloprost, or molsidomine, or superoxide dismutase on BP and ECG were meagre. A fall in BP was temporary and rarely exceeded 10% of the initial level.

DISCUSSION

Presently, it has been confirmed that iloprost, a prostacyclin analogue, executes cardioprotective action in experimental myocardial ischaemia by reducing the frequency of premature ventricular contrations, expanding a period of the onset of postischaemic arrhythmias and elongating the survival time of the affected animals, however, iloprost is unable to reduce the mortality caused by the ligation of the left descending coronary artery in cats. Indeed, in other animal species iloprost was reported to reduce the size of myocardial infarction, to enhance anatomical and functional recovery of ischaemic heart, to suppress postischaemic arrhythmias and to prevent thrombosis (16—18). In contrast with the efficacy of iloprost in models of experimental cardiac ischaemia, prostacyclin hardly has any therapeutical use in the treatment of patients with angina pectoris or myocardial infarction (4, 18).

On the other hand, molsidomine, a nitric oxide donor (12, 13), which is effective in ischeamic heart disease (19) and in thrombolysis of clots in coronary arteries of dogs and pigs (20), presently has been found worthless as a cardioprotective agent in the model of "no-reperfusion" myocardial ischaemia.

The main finding of this study is that a combination of ineffective molsidomine with effective iloprost is more efficient than iloprost alone in preventing the consequences of myocardial ischaemia. This "permissive" effect of molsidomine towards iloprost manifests itself by a dramatic reduction in the mortality rate and elongation of the survival time of animals following the occlusion of their left descending coronary artery. It has to be pointed out that the cardioprotection by a mixture of molsidomine and iloprost surpasses the cardioprotection by a megadose of superoxide dismutase.

The mechanism of the permissive cardioprotective action of a NO-donor towards the protective effect of an analogue of prostacyclin remains unknown. Recently, we have proposed that prostacyclin requires, for the development of its full- scale thrombolytic action, the permissive effect of nitric oxide (7, 11). *In vitro* nitric oxide hardly dissolves thrombi which are formed during the

interaction of decalcified blood with aspirinized endothelial cells while nitric oxide exerts a thrombolytic effect when endothelial cells are capable to generate prostacyclin. Nitric oxide also enhances thrombolysis by exogenous prostacyclin in the above system (7). A synergism between NO or NO-donors and PGI_2 or its analogues in their platelet-suppressant actions (5—8) and in their protective actions against ageing of leukocytes (7, 10) has been reported and it might be associated with intracellular interactions between the c-AMP and c-GMP generating systmes (21, 22). In patients with athersoclerosis the synergism between molsidomine and prostacyclin at a level of their fibrinolytic actions (11) and a synergism between isosorbide dinitrate and prostaglandin E_1 at a level of their anti-platelet actions (9, 23) are the examples of synergistic or permissive interactions between activators of guanylate and adenylate cyclases in clinical situations.

It might be alluded that lack of the expected beneficial effects of prostacyclin in the treatment of patient with ischaemic heart disease (4) is associated with deprivation of their coronary arteries of endogenous nitric oxide (24). Perhaps "permissive" effect of endogenous nitric oxide is indispensable for revealing of the cardioprotective action of exogenous prostacyclin? If so, there exists a sound experimental rationale for undertaking clinical trials with NO-donors and PGI_2-analogues in patients with ischaemic heart disease. The appeal of this proposal is emphasized by the fact that the enhancement of PGI_2 by NO is restricted to fibrinolysis, platelet-suppression and cardioprotection (present data) but it does not apply to their effects on vascular tone (7) and blood pressure (11, present data). Arterial hypotension is a major unwanted effect during the therapy with either molsidomine (19) or prostacyclin (4). Thereby, it is important to remember that looking for a synergism between NO and PGI_2 in their thrombolytic and cardioprotective actions one will not come across potentiation of their circulatory side effects.

REFERENCES

1. Moncada S, Palmer RMJ, Higgs EA. The discovery of nitric oxide as endogenous nitrovasodilator. *Hypertension* 1988; 12: 365—372.
2. Ignarro LJ. Endothelium-derived nitric oxide: action and properities. *FASEB J* 1989; 3: 31—36.
3. Vanhoutte PM. Molsidomine is a donor of EDRF. *J Cardiovasc Pharmacol* 1989; 14: S135—140.
4. Gryglewski RJ, Botting RM, Vane JR. Prostacyclin: from discovery to clinical application. In: Cardiovascular Significance of Endothelium-Derived Vasoactive Factors (Rubanyi GM, Ed), Futura Publ Co Mount Kisco, New York 1991; pp. 3—38.
5. Radomski MW, Palmer RMJ, Moncada S. The antiaggregating properities of vascular endothelium: interaction between prostacyclin and nitric oxide. *Br J Pharmacol* 1987; 92: 639—646.
6. Bult H, Fret HRJ, Herman AG. Interaction between SIN-1 and prostacyclin in inhibiting platelet aggregation. *J Cardiovasc Pharmacol* 1989; 14: S120—123.

7. Gryglewski RJ, Korbut R, Trąbka-Janik E, Zembowicz A, Trybulec M. Interaction between NO-donors and iloprost in human vascular smooth muscle, platelets and leukocytes. *J Cardiovasc Pharmacol* 1989; 14: S124—128.
8. Lidbury PS, Antunes E, de Nucci G, Vane JR. Interactions of iloprost and sodium nitroprusside on vascular smooth muscle and platelet aggregation. *Br J Pharmacol* 1989; 98: 1275—1280.
9. Katzenschlager R, Weiss K, Rogatti W, Stelzeneder M, Sinizinger H. Interaction between prostaglandin E_1 and nitric oxide (NO). *Thromb Res* 1991; 62: 299—304.
10. Korbut R, Trąbka-Janik E, Gryglewski RJ. Cytoprotection of human polymorphonuclear leukocytes by stimulators of adenylate and guanylate cyclases. *Eur J Pharmacol* 1989; 165: 171—172.
11. Bieroń K, Grodzińska L, Kostka-Trąbka E, Gryglewski RJ. Prostacyclin and molsidomine synergise in their fibinolitic and anti-platelet actions in patient with peripheral arterial disease. *Wien Klin Wschr* 1992; 105/1: 7—11.
12. Bohn H, Schönafinger K. Oxygen and oxidation promote the release of nitric oxide from sydnonimines. *J Cardiovasc Pharmacol* 1989; 14: S6—S12.
13. Feelisch M, Ostrowski J, Noack E. On the mechanism of NO release from sydnonimines. *J Cardiovasc Pharmacol* 1989; 14: Suppl. 11: S13—22.
14. Gerzer R, Karrenbrock W, Siess W, Heim J. M. Direct comparison of the effects of nitroprusside, SIN-1 and various nitrates on platelet aggregation and soluble guanylate cyclase activity. *Thromb Res* 1988; 52: 11—21.
15. Teteson JE, Moncada S, Vane JR. Effects of prostacyclin (PGX) on cyclic AMP concentration in human platelets. *Prostaglandins* 1977; 13: 389—397.
16. Araki H, Lefer AM. Role of prostacyclin in the preservation of ischemic myocardial tissue in the perfused cat heart. *Cir Res* 1980; 47: 757—763.
17. Schrör K, Smith IIEF, Lefer AM. The cats as an vivo model for myocardial ischemia and infarction. In: Progress in Pharmacology (Gustav Fisher Verlag, Stuttgart New York) 1988; Vol 6/4: pp. 31—91.
18. Muller B, Witt W, McDonald FM. Iloprost: Stable prostacycline-analoque. In: Cardiovascular Significance of Endothelium-Derived Vasaoactive Factors (Rubanyi GM, Ed), Futura Publ Co Mount Kisco, New York 1991; pp. 309—333.
19. Majid PA, DeFeyter PJF, Van der Wall EE, Warden R, Roos JP. Molsidomine in the treatment of patients with angina pectoris. Acute hemodynamic effects and clinical efficacy. *New Engl J Med* 1980; 302: 1—7.
20. Just M, Martorana PA. Effect of molsidomine on thrombus formation in stenosed coronary artieries of dogs and pigs. *J Cardiovasc Pharmacol* 1989; 14: S129—136.
21. Maurice DH, Haslam RJ. Molecular basis of the synergistic inhibition of platelet function by nitrovasodilators and activators of adenylate cyclase: inhibition of cyclic-AMP breakdown by cyclic-GMP. *Mol Pharmacol* 1990; 37: 671—681.
22. Bowen R, Haslam RJ. Effects of nitrovasodilators on platelet cyclic nucleotide levels in rabbit blood; role for cyclic AMP on synergistic inhibition of platelet function by SIN-1 and prostaglandin E_1. *J Cardiovasc Pharmacol* 1991; 17: 424—433.
23. Sinzinger H, Fitscha P, O'Grady J, Rauscha F, Rogati W, Vane JR. Synergistic effect of prostaglandin E_1 and isosorbit dinitrate in peripheral vascular disease. *Lancet* 1990; 335: 627—628.
24. Ganz P, Vekshtein VI, Yeung AC, et al. Impaired endothelial vasodilator function in human coronary arteries. In: Cardiovascular Significance of Endothelium-Derived Vasoactive Factors (Rubanyi GM, Ed), Futura Publ Co Mount Kisco, New York 1991; pp. 113—124.

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